

CLAIMS

What is claimed:

1. A method assaying for the presence of oxidative stress in a patient comprising the steps of:

(i) providing a predetermined amount of protein from a patient bound to a support forming a support-bound tissue protein;

(ii) reacting the support-bound tissue protein with 2,4-dinitrophenylhydrazine (DNPH) to form a derivatized support-bound tissue protein;

(iii) contacting the derivatized support-bound tissue protein with anti-DNPH antibody and maintaining that contact for a time period sufficient to form an immunocomplex between the derivatized support-bound tissue protein and the anti-DNPH antibody; and

(iv) determining the amount of immunocomplex present and comparing that amount to the amount of immunocomplex present in the same quantity of a standard sample, the amount greater than that present in the standard sample in excess of experimental error indicating the presence of oxidative stress in the patient.

2. The method of claim 1 wherein the support is a membrane.

3. The method of claim 1 wherein the support is a gel matrix.

4. The method of claim 1 wherein the support is a porous particle.

5. The method of claim 1 wherein the support is a plastic surface.

6. The method of claim 1 wherein the support is a biological molecule.

7. The method of claim 1 wherein the support is a nucleic acid.

8. The method of claim 1 wherein the support is a protein.

9. The method of claim 1 wherein the amount of immunocomplex present is determined by ultra-violet spectroscopy.

10. The method of claim 1 wherein the amount of immunocomplex present is determined by radiography.

11. The method of claim 1 wherein the amount of immunocomplex present is determined by fluorescence spectroscopy.

12. The method of claim 1 wherein the amount of immunocomplex present is determined by binding the immunocomplex to a second antibody and measuring the amount of bound secondary antibody.

13. The method of claim 12 wherein the second antibody is labeled with a fluorescent tag.

14. The method of claim 12 wherein the second antibody is labeled with a radioactive molecule.

15. The method if claim 12 wherein the second antibody is labeled with an indicator enzyme.

16. A method assaying for the presence of oxidative stress in a patient comprising the steps of:

(i) providing a predetermined amount of protein from a patient bound to a support forming a support-bound tissue protein;

(ii) reacting the membrane-bound tissue protein with 2, 4, dinitrophenylhydrazine (DNPH) to form a derivatized membrane-bound tissue protein;

(iii) contacting the derivatized membrane-bound tissue protein with anti-DNPH antibody and maintaining that contact for a time period sufficient to form an immunocomplex between the derivatized membrane-bound tissue protein and the anti-DNPH antibody; and

(iv) determining the amount of immunocomplex formed wherein the amount formed is determined by binding the immunocomplex to a second antibody that is labeled with horseradish peroxidase and measuring the amount of bound secondary antibody labeled with horseradish peroxidase and comparing that amount to the amount of bound secondary antibody labeled with horseradish peroxidase present in the same quantity of a standard sample, the amount greater than that present in the standard sample in excess of experimental error indicating the presence of oxidative stress in the patient.

17. A method assaying for the presence of oxidative stress in a patient comprising the steps of:

(i) providing a predetermined amount of protein from a patient bound to a support forming a support-bound tissue protein;

ii) reacting the support-bound tissue protein with 2,4-dinitrophenylhydrazine (DNPH) to form a derivatized support-bound tissue protein;

(iii) contacting the derivatized support-bound tissue protein with anti-nitrotyrosine antibody and maintaining that contact for a time period sufficient to form an immunocomplex between the derivatized support-bound tissue protein and the anti-nitrotyrosine antibody; and

(iv) determining the amount of immunocomplex present and comparing that amount to the amount of immunocomplex present in the same quantity of a standard sample, the amount greater than that present in the standard sample in excess of experimental error indicating the presence of oxidative stress in the patient.

18. The method of claim 17 wherein the support is a membrane.

19. The method of claim 17 wherein the support is a gel matrix.

20. The method of claim 17 wherein the support is a porous particle.

21. The method of claim 17 wherein the support is a plastic surface.

22. The method of claim 17 wherein the support is a biological molecule.

23. The method of claim 17 wherein the support is a nucleic acid.

24. The method of claim 17 wherein the support is a protein.

25. The method of claim 17 wherein the amount of immunocomplex present is determined by ultra-violet spectroscopy.

26. The method of claim 17 wherein the amount of immunocomplex present is determined by radiography.

27. The method of claim 17 wherein the amount of immunocomplex present is determined by fluorescence spectroscopy.

28. The method of claim 17 wherein the amount of immunocomplex present is determined by binding the immunocomplex to a second antibody and measuring the amount of bound secondary antibody.

29. The method of claim 28 wherein the second antibody is labeled with a fluorescent tag.

30. The method of claim 28 wherein the second antibody is labeled with a radioactive molecule.

31. The method if claim 28 wherein the second antibody is labeled with an indicator enzyme.

32. A method assaying for the presence of oxidative stress in a patient comprising the steps of:

(i) providing a predetermined amount of protein from a patient bound to a support forming a support-bound tissue protein;

(ii) reacting the membrane-bound tissue protein with 2, 4, dinitrophenylhydrazine (DNPH) to form a derivatized membrane-bound tissue protein;

(iii) contacting the derivatized membrane-bound tissue protein with anti-nitrotyrosine antibody and maintaining that contact for a time period sufficient to form an immunocomplex between the derivatized membrane-bound tissue protein and the anti-nitrotyrosine antibody; and

(iv) determining the amount of immunocomplex formed wherein the amount formed is determined by binding the immunocomplex to a second antibody that is labeled with horseradish peroxidase and measuring the amount of bound secondary antibody labeled with horseradish peroxidase and comparing that amount to the amount of bound secondary antibody labeled with horseradish peroxidase present in the same quantity of a standard sample, the amount greater than that present in the standard sample in excess of experimental error indicating the presence of oxidative stress in the patient.